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RONTGEN RESULT OF CARIES MOLAR IN WHITE RAT (*Rattus norvegicus*) WITH TREATMENT OF HERBAL TOOTH PASTE (*Pluchea indica* LEAVES EXTRACT)

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Dental caries is an infectious disease resulting email and dentin demineralization. Dental caries have relation with consumption of cariogenic food. But, dental caries also can be initiation with bacteria from *Streptococcus* class like *Streptococcus mutans*. *Beluntas* leaves (*Pluchea indica* L.) are a tropical herbal plant and this plant very easy to find and spread in Asia-Pacific areas. This plant is reported contain of anti-inflammation substance and decrease of necrosis in ventriculus, neutralization of snake toxin, antioxidants, antiulcerative, hepatoprotective, antimoeba, and broad spectrum of antimicrobial. The purpose of this research is to know differential Rontgen of caries molar in white rat (*Rattus norvegicus*) after treatment by herbal tooth paste of *Pluchea indica* leaves. First weeks of research, molar rat's induced by *Streptococcus mutans* and given by caries diet. In 9th and 10th weeks group 1 given 40% *Pluchea indica* leaves extract paste, group 2 given 50% *Pluchea indica* leaves extract paste, group 3 given 60% *Pluchea indica* leaves extract paste. After that, rats were necropsy and collected the pathological changes by Rontgen. Based on rontgen result, there was a difference of contrasting colors and shows the difference between the experimental groups. Results of Rontgen show there are differences in the color analysis especially in group 2 (50% *Pluchea indica* leaves extract paste) and shown similarity to the negative control group. This happen because of *Pluchea indica* extract paste can reduce amount of bacteria that initiated dental caries.

Keywords: caries, *Streptococcus mutans*, Rontgen, *Pluchea indica* leaves, extract tooth paste

1. INTRODUCTION

During the past decades the common consensus from many reports worldwide was that dental caries had declined significantly and was continuing to decline in populations (Bagramian *et al.* 2009). Dental caries, also known as tooth decay, affects the vast majority of adults and 60-90% of children in industrialized countries. Dental caries is a dynamic diet-microbial disease involving cycles of demineralization and remineralization (Ozdemir, 2014). This disease related to lower oral health and has been shown effect of individual performance. The most obvious is pain and discomfort, negative aesthetic appearance of caries and impact children's growth (Chambers. 2012). Dental caries in children and untreated, leads to pain, development of dentin facial anomalies and other serious health problems, such as severe toothache, dental abscess, destruction of bone, and spread of infection via the bloodstream (Bagramian *et al.* 2009).

The main factors that can causes dental caries are a) cariogenic bacteria, b) fermentable carbohydrates c) a susceptible tooth and host and d) time. In young children bacterial flora and host defenses are in the process of being developed tooth surfaces are newly erupted and may show hypo-plastic defects (Harris *et al.* 2004). Sugar consumption has been identified as the major cause of dental caries (Chambers. 2012). The most cariogenic sugar is Sucrose. Sucrose is highly soluble and diffuses easily into dental plaque, acting as a substrate for the production of extracellular polysaccharides and acids (Ozdemir, 2014). Actually, dental plaque forms continuously on tooth surfaces, and when exposed to fermentable carbohydrates, bacteria in the plaque create acid. Acid lowers the pH of the mouth, and a process of demineralization occurs on the enamel of the teeth (Chambers. 2012). Advanced caries results in cavitation, and can progress to the dentin and into the pulp chamber ultimately causing necrosis and periapical abscesses (Ozdemir, 2014).

Streptococcus mutans are considered to be the main bacteria that cause caries disease. Virulence factors of *Streptococcus mutans* are prevalent plaque adhesion-like cell surface proteins, acid tolerance, acid production and production of glucosyl transferases, mutacin and intracellular polysaccharides. *Streptococcus mutans* ferment many different sugars and they appear to metabolize sucrose to lactic acid more rapidly than other oral bacteria (Ozdemir, 2014).

Pluchea indica is a perennial shrub plant with small branches (0.5-2 m tall) widely found in warm temperature regions of countries such as Malaysia, Indonesia, Australia, Taiwan, India, and Mexico. *Pluchea indica* leaves are described as simple, sessile, glabrous, obovate, serrated with an acute apex. *Pluchea indica* leaves are 1-5 cm wide and 2-9 cm long. Flowering of *Pluchea indica* occurs at terminal or axillary corymbs (Suriyaphan. 2014).

Pluchea indica using for herbal medicine such as deodorize, drug for fever, drug for cough and anti-diarrhea. Usually stewed of *Pluchea indica* using to drug for skin (Winarno *et al.* 1988). Information indicates that *Pluchea indica* has potential as antimicrobial substance (Ardiansyah *et al.* 2003). *Pluchea indica* contain of phenol hydroquinone and tannin (active compound). Phenol hydroquinone and tannin has antimicrobial activity. Phenol hydroquinone substance become antimicrobial substance because contain of hydroxyl group (OH), ketone group (CO), and methoxide group (OCH₃) (Nishina *et al.* 1991 and Bisignano *et al.* 2000) while, the tannin as an antimicrobial substance because contain of hydroxyl group (Sakanaka *et al.* 1989). *Pluchea indica* contain flavonoid, tannin, sterol and phenol hydroquinone (Utami *et al.* 2013). Flavonoid is substance that comes from herbal plants. Flavonoid is a potential for antioxidant (Soeharto. 2012). Because of that compound, *Pluchea indica* potentially as herbal plant that can prohibit growth of *Streptococcus mutans*. Application of dental caries prevention is herbal toothpaste innovation and *Pluchea indica* herbal toothpaste is more nature than common toothpaste. The purpose of this research is to know the potential of *Pluchea indica* herbal tooth paste to prevent dental caries in *Rattus norvegicus* from Rontgen examination.

2. RESEARCH METHODOLOGY

2.1. Material of Plant and Making of Tooth Paste

Pluchea indica leaves gotten from Kalangan, Baturetno, Banguntapan, Bantul, *Pluchea indica* is known as herbal medicine. This species was identified and extracted in Pharmaceutical Faculty of Pharmacy, Gadjah Mada University. Medium extraction used *Dimethyl sulfoxide* substance.

The step of making tooth paste was done in Faculty of Pharmacy, Gadjah Mada University. Tooth paste made by concentration 40%, 50%, and 60%. These concentrations are equals with the Minimum Inhibitory Concentration (MIC).

2.2. Giving Feed Rats

Rats were given caries diet and base diet. Caries diet contain of 20% sucrose and 80% base diet (Br 2). Rats were feed base diet in negative control group. The group 1, group 2, group 3 and positive group given caries diet. Base diet and caries diet given for 10 weeks during research.

2.3. Bacterial Induction

The strain of bacteria used in this research is *Streptococcus mutans* from Microbiology Laboratory in Faculty of Veterinary Medicine Gadjah Mada University. This bacteria growth in Todd Hewitt Broth agar ([Oxoid™](#), Canada) in temperature of 37°C and anaerobe conditions using candle jar in two days incubation. The bacteria that already growing up then diluted using McFarland standard (10^8 CFU ml⁻¹). This dilution was using bacteria which induced sterile cotton bud. The dilution was swap into molar teeth surface. Induction was done every day in first week of research.

2.4. Giving *Pluchea indica* Extract Toothpaste

Rats were given the *Pluchea indica* extract toothpaste in nine weeks and ten weeks of research. Group treatment 1 was given 40% concentration of extract, second group 50% concentration and third group was giving 60% *Pluchea indica* extracts. *Pluchea indica* extract paste given once a day and given per oral use cotton bud sterile. The cotton bud sterile dipped on *Pluchea indica* extracts with various concentrations (depend on grub 1, 2 and 3) and rub it on molar teeth of rats.

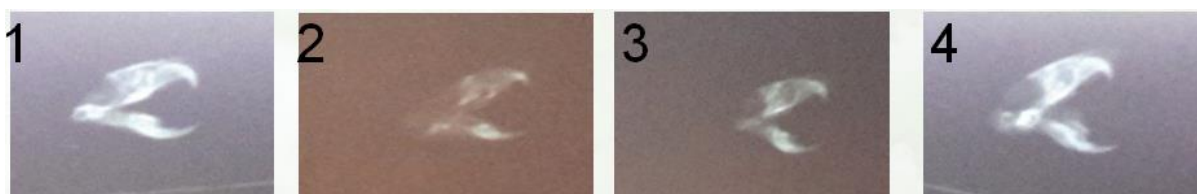
2.5. Taken Rontgen Pictures

All rats were euthanasia by chloroform and rats were necropsy at the end of research (the end of 10th weeks). Tooth and the mouth cavity fixed by 10% formalin. Then, tooth and the mouth cavity took for Rontgen pictures. Then Rontgen picture was analyzed.

3. RESULTS AND DISCUSSIONS

3.1. Dental Rontgen Result

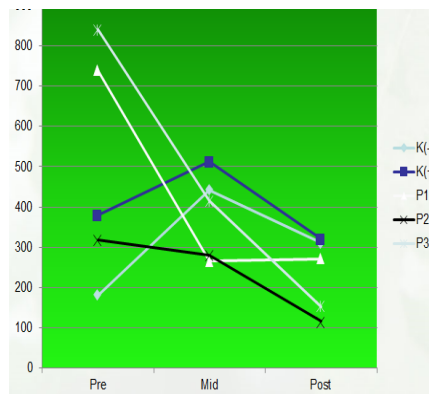
Dental Rontgen result after given treatment of *Pluchea indica* extract tooth paste shown in Picture 1.



Picture 1. Dental Rontgen after given treatment

Dental Rontgen result from negative control (normal group) show white calcification (number 1). Group of positive control is group that already caries without treatment of *Pluchea indica* extract tooth paste), shown decay of teeth and look opaque color (number 2). Group 3, this

group given treatment of 40% *Pluchea indica* extract tooth paste and shown most teeth still appears opaque (number 3). Group 4, this group given treatment of 50% *Pluchea indica* extract tooth paste and shown white calcification similarity with negative control and without any sign which could have been sequelae of dental caries (number 4).



Graphic 1. Total Plate Count (beginning treatment by middle and the end of treatment by *Pluchea indica* extract toothpaste)

During this research also discovered that total bacteria in molar teeth of rats were decrease after treatment by *Pluchea indica* extract paste. It can happen because of the compound of antimicrobial substance in *Pluchea indica* destroy the *Streptococcus mutans*. They was interfered the character hydrophobicity, GTFase, acidogenic, and aciduracities that bacteria. Reducing the Sucrose-independent (SI) adherence could reduce the character of disability bacteria in fusing with host cell. GTPase was playing role in change sucrose to glucan that induce the fusing bacteria into surface teeth. Character acidogenic could reduce with degradation of pH environment until glycolytic enzyme pressuring the product of acidic substance.

Acidity was causing by F- ATPase proton pump could stabilize of pH until crossing membrane until alkali condition in cytoplasm. *Pluchea indica* did not only bacteriostatic but also bactericidal. There was changing physiologies bacteria's cell membrane with environmental condition pass thought raising pKa until dying cell (Kabir *et al.* 2014). This research was investigating the rontgen result of caries molar in white rat (*Rattus norvegicus*) with treatment of herbal tooth paste (*Pluchea indica* leaves extract). From the Rontgen result and analyzes amount of bacteria, *Pluchea indica* extract tooth paste can reduce of bacteria virulence in mouth that initiated dental caries. So, *Pluchea indica* extract toothpaste having potential agent for treatment caries in rat tooth. This reference also can be research more effect in human.

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